Influence of autogenous platelet concentrate on combined GTR/graft therapy in intrabony defects: a 13-year follow-up of a randomized controlled clinical split-mouth study

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Abstract

Aim: To investigate the clinical long-term outcomes 13 years following guided tissue regeneration (GTR) in deep intrabony defects with and without additional application of autogenous platelet concentrate (APC).

Methods: In 25 patients, two deep contra-lateral intrabony defects were treated according to GTR using ß-TCP and bio-resorbable membranes. In test defects, APC was applied additionally. After 13 years, clinical healing results were assessed and compared to results at baseline and after 1 year. Furthermore, a tooth survival analysis was carried out.

Results: After 13 years, 22 patients were available for tooth survival analysis showing 81.8% of test and 86.4% of control teeth still *in situ*. Based on the 15 patients still available for split-mouth analysis, median CAL was 10.0 mm in test and 12.0 mm in control sites at baseline. After 1 year, both groups revealed significant CAL gains of 5.0 mm, followed by a new CAL loss of 1.0 mm in the following 12 years. There were no significant differences between test and control sites.

Conclusion: Within the limits of this study, the data shows that most of the CAL gain following GTR can be maintained over 13 years. The additional use of APC had no positive influence on the long-term stability.

Clinical relevance

Scientific rationale for the study

To investigate the long-term outcomes 13 years after combined GTR/graft therapy with additional application of autogenous platelet concentrate (APC) in deep intrabony defects.

Principal findings

After 13 years, more than 80% of teeth were still *in situ*. Significant CAL gains were found after 1 and 13 years as compared to baseline irrespective of additional APC-application. However, a new attachment loss of 1 mm occurred between 1 and 13 years.

Practical implications

The clinical parameters after GTR/graft therapy can be maintained over a period of 13 years, while additional application of APC showed no additional benefit.

Introduction

Guided tissue regeneration (GTR) is a widely accepted treatment concept for regenerative periodontal therapy by applying cell-occlusive membranes, which act as physical barriers preventing epithelial down-growth and maintaining a space for the slowly migrating cells of the periodontal ligament (Caffesse et al. 1988; Karring et al. 1993; Larsson et al. 2016). For the treatment of wide, non-containing intrabony defects, GTR is often combined with bone grafts, which stabilize the blood-clot and support the muco-periostal flap and the barrier membrane preventing a collapse into the defect (Cortellini & Tonetti 2015; Needleman et al. 2006; Reynolds et al. 2014; Sculean et al. 2008b).

However, there is still a high variability with regard to clinical outcomes after regenerative periodontal therapy due to several influence factors related to the individual patient, the defect or the surgical technique used (Cortellini & Tonetti 2000, 2015). Among patient-related factors, the innate wound healing potential of the individual patient is of vital importance for the healing outcomes (Kornman & Robertson 2000) and is determined by the presence, amount and balance of growth factors crucial for periodontal wound healing, such as platelet-derived growth factor (PDGF) or transforming growth factor-β (TGF-β) (Smith et al. 2015).

Autogenous platelet concentrate (APC), often also referred to as platelet-rich plasma (PRP) (Marx, 2001), had been suggested as a natural, patient-own source of these relevant growth factors, as the α-granules of thrombocytes contain PDGF, TGF-β, and insulin-like growth factor (Bosshardt et al. 2015; Christgau et al. 2006a; Smith et al. 2015). APC had first been introduced in the field of oral and maxillofacial surgery in 1997 by Whitman *et al.* (Whitman et al. 1997), while Marx *et a*l. had been the first to propose the use of PRP for the modulation of bone healing (Marx et al. 1998). Since then, several clinical studies have been published investigating the benefits of additional application of APC in regenerative periodontal therapy in combination either with GTR (Camargo et al. 2002; Cetinkaya et al. 2014), grafts (Demir et al. 2007; Hanna et al. 2004; Okuda et al. 2005; Yassibag-Berkman et al. 2007) or combined GTR/graft therapy (Camargo et al. 2005, 2009; Christgau et al. 2006b; Döri et al. 2007a, 2007b, 2008; Moder et al. 2012).

In a prospective clinical split-mouth study from our group, the impact of additional application of APC on combined GTR/graft therapy with β-tricalcium phosphate (β-TCP) granules and a bio-resorbable, polylactic/polyglycolic acid copolymer (PLA/PGA) GTR membrane was investigated in deep intrabony defects (Christgau et al. 2006b). Apart from a tendency to less membrane exposures and an initially accelerated bone density gain during the first 6 months, no relevant effects on the early regeneration outcomes were found after 6 and 12 months (Christgau et al. 2006b). In contrast, after 7 years clinical results suggested even a possibly negative influence of APC showing less long-term stability of the clinical healing outcomes in test sites compared to the control sites without the additional application of APC (Moder et al. 2012). To the best of our knowledge, there has been no study so far investigating the long-term results of more than 7 years following combined GTR/graft therapy with additional application of APC. Furthermore, Wu *et al.* recently concluded in their systematic review on the long-term efficacy of periodontal regenerative therapy that there is still an urgent need for more long-term data (Wu et al. 2017).

Consequently, the aim of this follow-up study was to evaluate the long-term outcomes 13 years after combined GTR/graft therapy with and without additional application of APC.

Material and Methods

Study design

The present study is a 13-year follow-up of a controlled randomized prospective clinical split-mouth study investigating the influence of additional application of autogenous platelet concentrate (APC) on the healing results in deep intrabony periodontal defects following guided tissue regeneration (GTR) combined with β-TCP (Christgau et al. 2006b). The study design followed the requirements outlined in the CONSORT 2010 statement (Moher et al. 2010) and was approved by the ethics committee of the University of Regensburg in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. After detailed description of the proposed treatments written informed consent was obtained from all individual participants included in the study.

Patient selection

The study originally included 25 systemically healthy patients recruited from the patient pool of the Department of Conservative Dentistry and Periodontology of the University Medical Center Regensburg. For inclusion in this study, all patients had to have one pair of contra-lateral deep intrabony, interproximal periodontal defects with a probing pocket depth (PPD) of at least 6 mm and radiographic evidence of angular bone loss of at least 4 mm at baseline. None of the teeth showed furcation involvement. Full-mouth supra- and subgingival scaling and root planing as well as splinting of highly mobile teeth had to be successfully completed at least 4 to 6 weeks before surgery. None of the patients suffered from a systemic disease with a possibly negative influence on healing outcomes.

Clinical therapeutic procedures

The APC was prepared as described earlier in detail (Christgau et al. 2006a, 2006b). In brief, APCs were prepared at the Department of Transfusion Medicine of the University Medical Center Regensburg using an apheresis technique. For clinical application, 2.5 ml APC was reactivated with 0.5 ml of a sterile 10% calcium chloride solution. All surgical interventions

were performed by one experienced surgeon (MC) according to the principles of GTR therapy and have been described in detail earlier (Christgau et al. 2006b). Allocation of treatment sites to test and control groups was performed by means of a computer-generated randomization table created by a mathematician (KAH).

Both defects in each patient were treated in the same session. Following sulcular incisions, buccal and oral muco-periosteal flaps were elevated. After debriding the defects thoroughly, test sites were treated as follows: root surfaces were conditioned with 24% EDTA gel (Prefgel; Straumann, Basel, Switzerland) for 2 min. After rinsing with sterile 0.9% sodium chloride solution, reactivated APC (Christgau et al. 2006a, 2006b) was applied to the root surfaces. Subsequently, the defects were filled with β-TCP granules (Ceros; Mathys, Bettlach, Switzerland), which had been soaked in APC, and covered with a bio-resorbable PLA/PGA membrane (Resolut XT; Gore Medical, Flagstaff, AZ, USA). The surgical sites were closed tension-free by coronally repositioned flaps. Control sites were treated accordingly without applying APC, whereby the β-TCP granules had been soaked with patient blood taken from the surgical site. Post-operative procedures were as described earlier (Christgau et al. 2006b).

Within the first post-operative year, all patients were scheduled in a strict supportive periodontal therapy (SPT) program at the Department of Conservative Dentistry and Periodontology of the University Medical Center Regensburg with visits every 3 months. After that period, further participation in the SPT program was highly recommended but most of the patients returned to their referring dentists. The remaining patients were scheduled twice a year for SPT in the undergraduate program of the Department of Conservative Dentistry and Periodontology.

Clinical examination

Clinical examination was performed by two blinded examiners (FC; LT), who had been calibrated to the principal investigator (MC) in advance. The examiners were not involved in the treatments and not aware of the treatment modality used in the individual defects.

The clinical parameters were recorded immediately before as well as 1 and 13 years after periodontal surgery. Oral hygiene was measured by means of the full-mouth approximal plaque index (API) (Lange et al. 1977), gingival inflammation by means of the papillary bleeding index (PBI) (Saxer & Mühlemann, 1975).

The subsequent clinical parameters were recorded for assessment of the healing results following regenerative therapy using a pressure-calibrated probe: gingival recession (REC) as the distance between cemento-enamel junction (CEJ) or the margin of a restauration to the gingival margin, probing pocket depth (PPD) as the distance from the gingival margin to the fundus of a periodontal pocket, and clinical attachment level (CAL) as the distance from the CEJ or the margin of a restauration to the fundus of a periodontal pocket. Furthermore, bleeding on probing (BOP) was measured. CAL change was determined to be the primary outcome variable. In addition, the vertical relative attachment gain (V-rAG) was calculated as the percentage of the CAL gain related to the BL depth of the osseous defect measured intra-operatively (Christgau et al. 2006b).

Compliance

For evaluation of the patients' compliance, the regularity of participation in supportive periodontal therapy (SPT) was calculated based on the dental chart of each individual patient. A patient who attended SPT at the Department of Conservative Dentistry and Periodontology of the University Medical Center Regensburg at least once per year was classified to have 'regular SPT'. Patients who failed no more than one year during the whole 13-year-period were considered to have 'irregular SPT'. Patients failing more often were classified to have 'no SPT' at the University Medical Center.

Data analysis

The single patient was regarded to be the statistical unit in this study. As discussed earlier (Christgau et al. 1997), clinical measurements were reported as median values (with 25%/75% percentiles) and were statistically evaluated using a non-parametric procedure.

Considering the paired nature of the split-mouth data, the Wilcoxon-Signed-rank test was applied on a significance level of α=0.05 using SPSS for Windows, version 23 (SPSS Inc., Chicago, IL, USA) (Woolson & Clarke, 2002).

Results

All initially recruited 25 patients received the intended treatment and completed the 1-year observation period (baseline to 12 months) (Christgau et al. 2006b). After 13 years, 3 patients were not available anymore. 21 of the remaining 22 patients could be scheduled for clinical examination, while the other patient was only available for an oral interview via telephone.

Due to tooth extractions by the referring dentists for prosthodontic reasons, only 15 patients were suitable for pair-wise split-mouth analysis after 13 years (Figure 1). A tooth survival analysis could be performed in 22 patients. Figure 2 exemplarily shows the radiographic healing in a control site at baseline as well as 1 and 13 years following regenerative therapy.

Compliance with SPT

The calculation of the SPT frequencies was carried out based on the dental charts of the 22 patients who were available for tooth survival analysis. 23% of the patients had participated regularly in SPT, 18% had participated irregularly, and 59% received no SPT at the University Medical Center.

Tooth survival analysis

Tooth survival was analyzed in 22 patients. After 13 years, 7 teeth in 6 patients had been extracted by the referring dentists due to prosthodontic reasons: 4 teeth in the test group and 3 teeth in the control group. Accordingly, 81.8% and 86.4% of the teeth were still *in situ* in the test or control group, respectively. No association of tooth loss could be found with regard to tooth type while there was a tendency for more tooth loss in initially deeper defects (6 out of 7 tooth losses occurred in teeth with CAL of 12.0 mm or more at baseline; Table 1). Furthermore, poor compliance with the participation in SPT seemed to have an impact on tooth loss as well: it occurred in 3 patients with no SPT, in 2 patients with irregular SPT, and only in 1 patient with regular SPT at the University Medical Center.

Clinical parameters

For split-mouth analysis, the clinical data of 15 patients was available. The median full-mouth API increased from 10% at baseline to 12% after 1 year and 25% after 13 years, while the median full-mouth PBI declined from 14% at baseline to 10% after 1 year and 4% after 13 years. These differences were not statistically significantly different. The median PBI at the surgical sites was 1.0 at baseline in both, test and control sites, decreased to 0.0 after 1 year, and remained stable after 13 years. The median BOP was positive (bleeding) in both groups at baseline, negative (no bleeding) after 1 year and positive again after 13 years.

Both groups showed a median baseline PPD of 10.0 mm, which decreased by 6.0 mm (both groups) to 3.0 mm (test) or 4.0 mm (control), respectively, after 1 year. After 13 years, median PPD increased again by 1.0 mm to 5.0 mm (both groups). The median REC started at baseline at 1.0 mm (test) or 2.0 mm (control) and increased to 3.0 mm (both) after 1 year, declining again in test group to 2.0 mm while remaining stable in the control group after 13 years. The median CAL at baseline was found to be 10.0 mm in test sites and 12.0 mm in control sites and improved by 5.0 mm to 6.0 mm in both groups after 1 year. After 13 years, the median CAL increased again by 1.0 mm to 7.0 mm in both groups. The vertical relative attachment gain after 1 year was found to be 75% in the test and 76.9% in the control group and decreased to 50% in the test and 66.7% in the control group after 13 years. No significant differences between test and control sites were found. The results of the clinical examination are summarized in Tables 2 and 3. Additionally, Table 4 shows the frequency distribution of sites with PPD ≤ 3 mm, 4-5 mm as well as ≥ 6 mm for all test and control sites at baseline as well as after 1 and 13 years.

Discussion

The present study investigated the long-term healing outcomes 13 years after combined GTR/graft therapy with or without additional application of autogenous platelet concentrate. In general, studies reporting on long-term outcomes after regenerative periodontal therapies are scarce (Wu et al. 2017). To date, there are only five prospective randomized clinical trials on GTR or combined GTR/graft therapy in intrabony defects with follow-up for 10 years (Nickles et al. 2009; Nygaard-Østby et al. 2010; Pretzl et al. 2008, 2009; Sculean et al. 2008a) and only one very recent study on GTR with a longer observation period (*i.e.* 20 years) than in the present study (Cortellini et al. 2017). Furthermore, this is the first study investigating the impact of APC on the long-term clinical healing outcomes after combined GTR/graft therapy.

Clinical parameters

A general drawback of this kind of long-term studies is the fact that usually only a part of the initial patient population is still available. Accordingly, in the present study only 15 patients were still available for split-mouth analysis after 13 years, while in the original study 25 patients had been included, contributing test and control defects for split-mouth analysis each. Therefore, a reduced statistical power of the long-term data must be accepted and, consequently, the results have to be interpreted with caution. While in the original study on 25 patients test and control defects both showed a median baseline CAL of 10.0 mm (Christgau et al. 2006b), in this 13-year follow-up the baseline CAL was 10.0 mm in test and 12.0 mm in control sites. This can be explained by the selection of the 15 patients, who were still available for split-mouth analysis after 13 years. However, this difference in baseline CAL was not statistically significant.

In the present study, the median CAL gain 1 year after periodontal surgery measured 5.0 mm in both groups. These results were in the upper range compared to those of previous studies on GTR therapy with bio-resorbable membranes in intrabony defects (Murphy & Gunsolley 2003), where at best a mean CAL gain of 4.60 mm was reported 1 year after GTR using

PLA/PGA membranes (Cortellini et al. 1996). The better outcomes in our study may be explained by the combination with graft material.

Studies reporting on long-term results after GTR or combined GTR/graft therapy with bio-resorbable membranes in intrabony defects found mean CAL gains in a range between 2.4 to 3.8 mm 10 years postoperatively (Nickles et al. 2009; Nygaard-Østby et al. 2010; Pretzl et al. 2008, 2009; Sculean et al. 2008a). Nygaard-Østby *et al.* observed a mean CAL gain of 3.8 mm 10 years after GTR therapy with bio-resorbable polylactide (PLA) membranes combined with autogenous bone graft (Nygaard-Østby et al. 2010). Our 13-year results revealed median CAL gains of 3.0 mm in the test and 5.0 mm in the control group, which were not statistically significantly different. Although these CAL gains are similar or slightly better as compared to the afore-mentioned long-term studies, we observed a new median CAL loss of 1.0 mm in both groups between the 1-year and the 13-year follow-up. This is also reflected in the frequency distribution of sites with PPD ≤ 3 mm, 4-5 mm as well as ≥ 6 mm at the 3 examination time points: while after 1 year all sites showed PPD of maximum 5 mm, after 13 years some sites worsened again (7 and 4 sites with PPD ≥ 6 mm for test and control group, respectively). These results are in line with the literature, where CAL changes ranged from a mean CAL loss of 1.62 mm between 1 and 10 years postoperatively (Pretzl et al. 2009) to a mean CAL gain of 1.2 mm between 9 months and 10 years after surgery (Nygaard-Østby et al. 2010). This additional CAL gain in the latter study was explained by a still not complete tissue maturation after 9 months (Nygaard-Østby et al. 2010). In the study with the longest follow-up period in the literature, Cortellini *et al.* found mean CAL losses of 0.1 mm or 0.5 mm dependent on the respective surgical technique (modified papilla preservation technique or conventional access flap, respectively) between 1 and 20 years after GTR therapy using expanded polytetrafluoroethylene (ePTFE) membranes (Cortellini et al. 2017).

Tooth loss and compliance

Hujoel *et al.* suggested to use true end points (*e.g.* tooth loss) in addition to surrogate

parameters (*e.g.* CAL changes) for evaluation of distinct periodontal treatment approaches (Hujoel et al. 2000). In this clinical trial, 13 years after periodontal surgery 81.8% and 86.4% of the teeth were still *in situ* in the test or control group, respectively. Consequently, 7 teeth (test: 4; control: 3) in 6 patients were lost during the study period. However, the decisions for tooth retention or extraction were made *alio loco* by the respective referring dentists. According to the patients' self-report, the tooth extractions were due to prosthodontic reasons (mostly in the context of more complex prosthetic rehabilitations) and thus may not be considered as periodontal complications.

It is well-known that regular participation in supportive periodontal therapy (SPT) is crucial for long-term success of periodontal treatment (Axelsson & Lindhe 1978; Axelsson et al. 2004). Cortellini & Tonetti found significantly more new attachment loss and tooth loss in cases without regular SPT in a cumulative long-term analysis up to 16 years following GTR (Cortellini & Tonetti, 2004). In the present study, all patients had to attend SPT every 3 months during the first year postoperatively. However, during the following 12 years, only 23% of the patients further participated in the SPT program at the University Medical Center on a regular basis (*i.e.* at least once per year) and 18% received SPT irregularly (*i.e.* maximum one year without SPT during the entire study period). In contrast, 59% of the patients did not further attend SPT at the University Medical Center during this period (*i.e.* two or more years without attendance). This may be reflected in the tooth survival analysis, where 5 out of the 6 patients with tooth loss showed no or irregular SPT attendance at the University Medical Center. However, due to the geographical context and the wide catchment area of the University Medical Center Regensburg many patients had to accept long ways accompanied by additional costs to reach the University Medical Center and most of them preferred to attend their local dentists after the 1-year follow-up. Return of the patients to their referring dentists does not necessarily mean insufficient professional maintenance. However, the quality of the maintenance could not be controlled by the investigators. Having this in mind, it is noteworthy that the oral hygiene parameters remained quite stable over the 13-year period with a slightly increasing full-mouth API (10% at

baseline, 12% after 1 year, 25% after 13 years) and even marginally decreasing full-mouth PBI (14% at baseline, 10% after 1 year, 4% after 13 years). Furthermore, it has to be emphasized that both, test and control sites, suffered equally from the low participation in SPT at the University Medical Center because due to the split-mouth design every patient served as his or her own control (Hujoel & Moulton 1988; Koch & Paquette 1997).

Impact of additional application of APC

As the aim of the original split-mouth study was to investigate the effects of APC on clinical healing outcomes after combined GTR/graft therapy, all influence factors were kept constant besides the additional application of APC. Therefore, a negative control group (*i.e.* access flap surgery) had not been included in this split-mouth study.

In the present study, no benefits of additional application of APC were found on long-term clinical healing outcomes following combined GTR/graft therapy. However, these results should be interpreted with caution as only 15 out of the original 25 were still available for split-mouth analysis after 13 years, wherefore a reduced statistical power must be accepted. Nevertheless, the results of this study are in accordance with some recent systematic reviews concluding that platelet concentrates may reveal a positive adjunctive effect on periodontal regenerative therapy outcomes in intrabony defects when combined with graft materials alone, but not in combination with GTR or combined GTR/graft therapy (Del Fabbro et al. 2011; Hou et al. 2016; Panda et al. 2016; Roselló-Camps et al. 2015). It was suggested that the proven efficacy of GTR could mask additional effects of platelet concentrates. When using graft materials without membranes, the dense fibrin network formed after platelet activation may act as a barrier and prevent epithelial migration into the defect, explaining the superior results in these cases (Del Fabbro et al. 2011; Panda et al. 2016).

In the 7-year follow-up of our study, it was found that the test sites exhibited worse clinical healing outcomes than the control sites in terms of a statistically significant greater increase in PPD and CAL loss from 1 to 7 years (Moder et al. 2012). After 13 years, we still found less median CAL gain between baseline and 13 years for test (3.0 mm) than control sites (5.0

mm), which was however not found to be statistically significant anymore. Likewise, the frequency distribution of sites with PPD ≤ 3 mm, 4-5 mm as well as ≥ 6 mm showed slightly more worsening of test sites after 13 years as compared to control sites. Although the baseline characteristics of test and control defects exhibited no statistically significant differences, there was a tendency for worse median CAL at baseline in control (12.0 mm) than in test defects (10.0 mm). This may explain the trend for better performance of control than test defects because it is well-known that defects with greater defect depth at baseline may have a better potential for periodontal regeneration than more shallow defects (Kornman & Robertson 2000).

Conclusions

- Most of the CAL gain found 1 year after combined GTR/graft therapy could be maintained over a period of 13 years, although there was some new CAL loss between 1 and 13 years.
- Within the limitations of this 13-year study, the application of APC showed no statistically significant benefits on the long-term clinical healing outcomes. This is in line with the 1-year results of the original study.

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Tables

Table 1. Frequency distribution of tooth loss in test and control sites according to tooth type and clinical attachment level (CAL) at baseline.

	test	control
Tooth type		
Incisors, canines	2	1
Premolars	2	0
Molars	0	2
Total	4	3
CAL at baseline		
10 mm	1	0
11 mm	0	0
12 mm	0	1
13 mm	3	1
14 mm	0	1
Total	4	3

n: number of lost teeth.

Table 2. Split mouth analysis: papillary bleeding index (PBI), bleeding on probing (BOP), gingival recession (REC), probing pocket depth (PPD) and clinical attachment level (CAL) at the surgical sites at baseline as well as after 1 and 13 years (median, 25%/75% percentiles).

	test sites (n=15)					**control sites** (n=15)				
	PBI	BOP*	REC [mm]	PPD [mm]	CAL [mm]	PBI	BOP*	REC [mm]	PPD [mm]	CAL [mm]
baseline										
median	1.0[2]	1.0[1,3]	1.0[1,2]	10.0[1,2]	10.0[1,2]	1.0	1.0	2.0[1,2]	10.0[1,2]	12.0[1,2]
25%	0.0	0.0	0.0	9.0	9.0	0.0	0.0	0.0	9.0	10.0
75%	1.0	1.0	3.0	10.0	12.0	2.0	1.0	3.0	10.0	13.0
1 year										
median	0.0	0.0[1]	3.0[1]	3.0[1,3]	6.0[1]	0.0	0.0[3]	3.0[1]	4.0[1]	6.0[1]
25%	0.0	0.0	1.0	3.0	4.0	0.0	0.0	1.0	3.0	5.0
75%	1.0	0.0	5.0	4.0	7.0	1.0	1.0	4.0	5.0	7.0
13 years										
median	0.0[2]	1.0[3]	2.0[2]	5.0[2,3]	7.0[2]	0.0	1.0[3]	3.0[2]	5.0[2]	7.0[2]
25%	0.0	0.0	2.0	3.0	5.0	0.0	1.0	2.0	3.0	6.0
75%	0.0	1.0	5.0	6.0	10.0	2.0	1.0	5.0	6.0	8.0

n: number of split-mouth defects;
*: 0=negative, 1=positive;
[+]: statistically significant difference between test and control ($p \leq 0.05$);
[1]: statistically significant difference between baseline and 1-year ($p \leq 0.05$);
[2]: statistically significant difference between baseline and 13-years ($p \leq 0.05$);
[3]: statistically significant difference between 1-year and 13-years ($p \leq 0.05$).

Table 3. Split mouth analysis: changes in gingival recession (REC), probing pocket depth (PPD), clinical attachment level (CAL) and vertical relative attachment gain (V-rAG) at the surgical sites after 1 and 13 years (median, 25%/75% percentiles).

	test sites (n=15)				**control sites** (n=15)			
	ΔREC [mm]	ΔPPD [mm]	ΔCAL [mm]	V-rAG [%]	ΔREC [mm]	ΔPPD [mm]	ΔCAL [mm]	V-rAG [%]
baseline – 1 year								
median	-1.0[2]	6.0[1,2]	5.0[2]	75[2]	-1.0	6.0[2]	5.0[2]	76.9[2]
25%	-2.0	5.0	4.0	66.7	-1.0	5.0	4.0	55.6
75%	0.0	7.0	5.0	83.3	0.0	6.0	7.0	87.5
baseline – 13 years								
median	-2.0	4.0[1,3]	3.0[3]	50[3]	-1.0[3]	5.0[3]	5.0[3]	66.7[3]
25%	-3.0	3.0	2.0	30	-2.0	4.0	3.0	33.3
75%	0.0	6.0	5.0	100	0.0	6.0	6.0	75
1 year – 13 years								
median	0.0[2]	-1.0[2,3]	-1.0[2,3]	-20[2,3]	0.0[3]	-1.0[2,3]	-1.0[2,3]	-12.5[2,3]
25%	-2.0	-3.0	-2.0	-33.3	-1.0	-2.0	-2.0	-25
75%	1.0	0.0	1.0	20	1.0	0.0	1.0	10

n: number of split-mouth defects;
[+]: statistically significant difference between test and control ($p \leq 0.05$);
[1]: statistically significant difference between (BL – 1 year) and (BL – 13 years) ($p \leq 0.05$);
[2]: statistically significant difference between (BL – 1 year) and (1 year – 13 years) ($p \leq 0.05$);
[3]: statistically significant difference between (BL – 13 year) and (1 year – 13 years) ($p \leq 0.05$).

Table 4. Split mouth analysis: Frequency distribution of PPD ≤ 3 mm, 4-5 mm as well as ≥ 6 mm at the surgical sites at baseline as well as after 1 and 13 years.

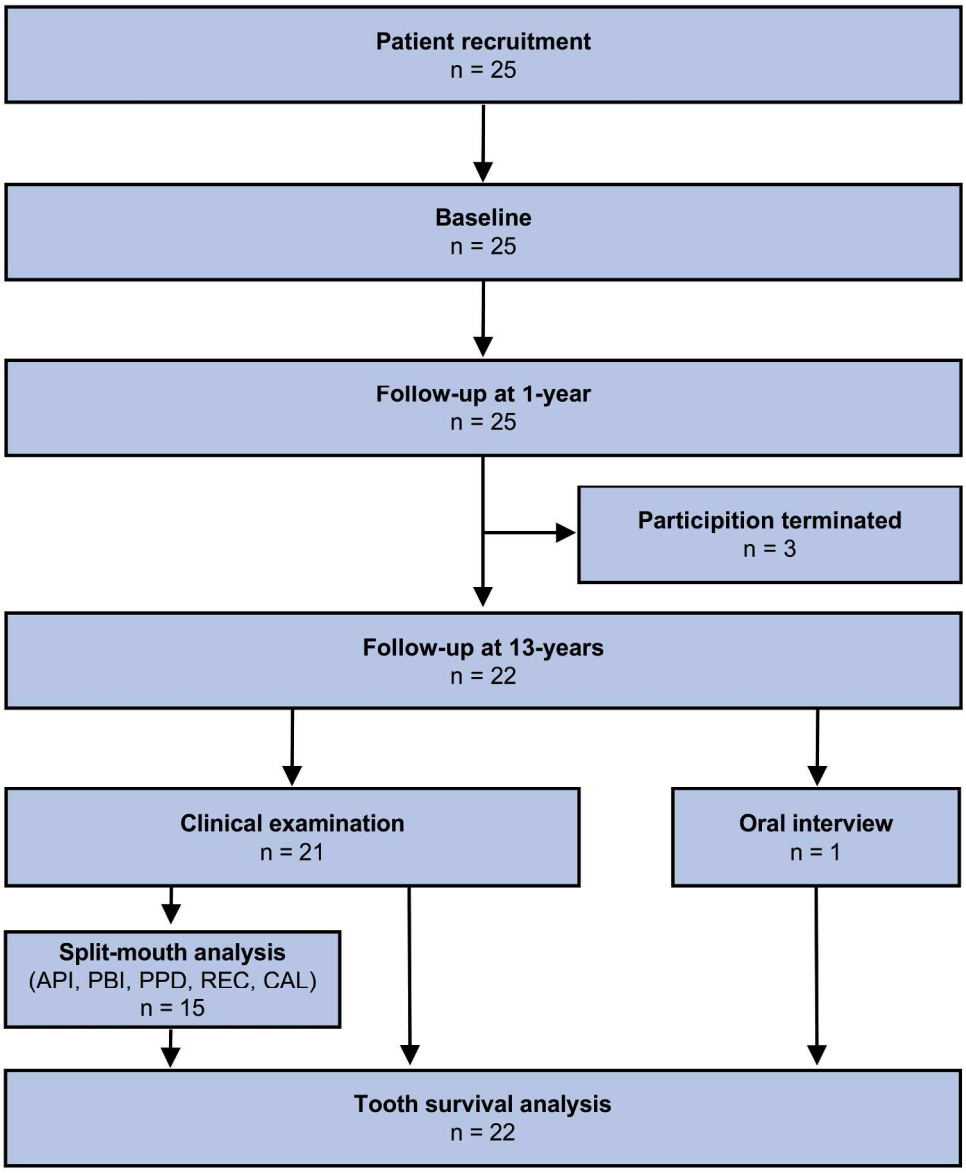
	test sites (n=15)			**control sites** (n=15)		
	≤ 3 mm	4-5 mm	≥ 6 mm	≤ 3 mm	4-5 mm	≥ 6 mm
baseline	-	-	15	-	-	15
1 year	8	7	-	6	9	-
13 years	4	4	7	4	7	4

n: number of split-mouth defects.

Figure Legends

Figure 1: Flowchart of the study outline.

Figure 2: Radiographic healing in a control site at baseline as well as 1 and 13 years after combined GTR/graft treatment without additional application of APC.



Flowchart of the study outline.‼ ┼

125x150mm (600 x 600 DPI)

Radiographic healing in a control site at baseline as well as 1 and 13 years after combined GTR/graft treatment without additional application of APC.

52x18mm (600 x 600 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6-8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a
Sample size	7a	How sample size was determined	8-9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6-7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	n/a
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	n/a
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	23-26
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n/a
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10-11
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	n/a
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	12-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-16
Other information			
Registration	23	Registration number and name of trial registry	n/a
Protocol	24	Where the full trial protocol can be accessed, if available	n/a
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	1, 17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.